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Supplementary Information for:

Nucleosomes inhibit target cleavage by CRISPR-Cas9 *in vivo*

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This PDF file includes:

Figs. S1 to S13
Tables S1 to S4
Reference for SI citation

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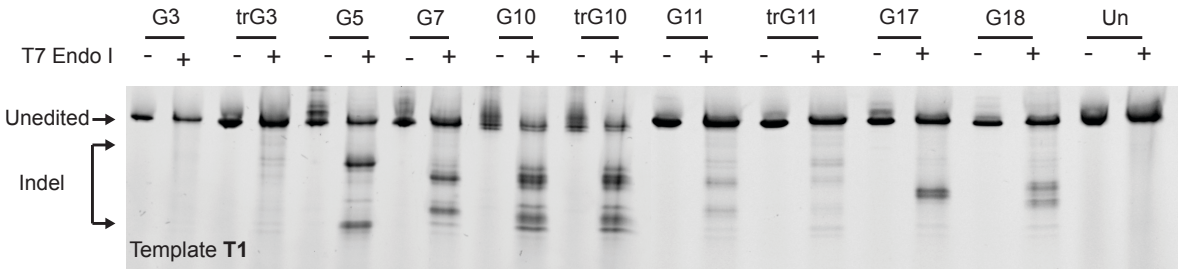


Rank	17	5	9	16	15	6	13	Broad
	14	15	8	1	2	3	12	ChopChop
	1	15	3	17	13	14	10	ATUM
	7	2	3	1	6	5	4	Subjective

Fig. S1. Ranking and analysis of sgRNAs for targets in human β-globin Exon 1. The top portion of the figure is a T7 endonuclease assay of Cas9 mutagenesis with each of the individual sgRNAs, reprinted with permission from ref. 1. sgRNAs with 20-nt guide sequences are labeled Gn, and truncated versions of some of these are labeled trGn. In the bottom portion, ranks assigned to the full-length sgRNAs by three on-line tools are tabulated. The web sites were given the full Exon 1 sequence. At the very bottom is a subjective assessment of the rank order of observed activities. The web searches were performed in January, 2017. Current web addresses are: Broad, https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design; ChopChop, http://chopchop.cbu.uib.no/; ATUM, https://www.atum.bio/eCommerce/cas9/input. Ranks assigned by the on-line tools do not agree well with each other, and none of them corresponds particularly well to the observed mutagenesis levels.

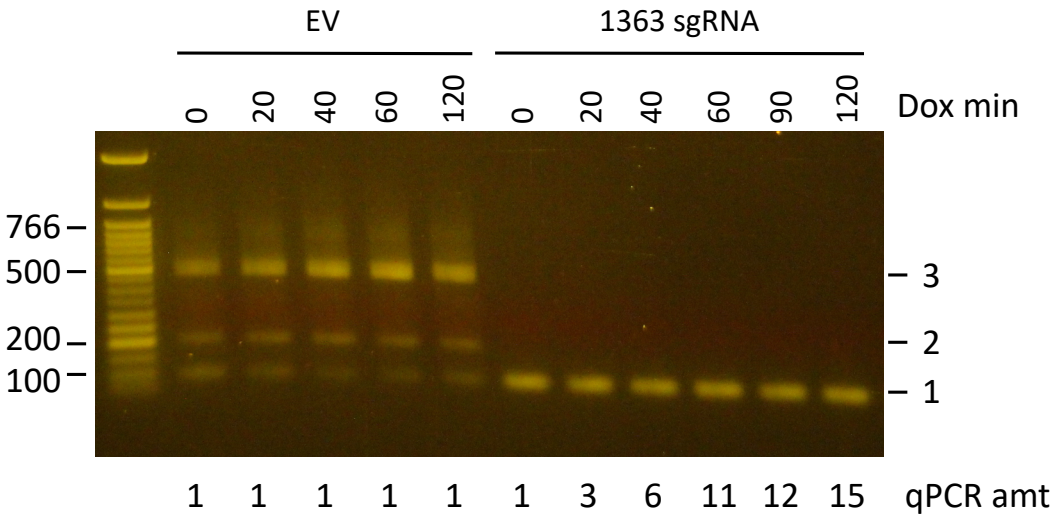


Fig. S2. Analysis of sgRNA expression. RNA was isolated at the indicated times after induction of sgRNA synthesis with doxycycline (Dox min) and subjected to strand-specific qPCR, using primers for the *HO* 1363 sgRNA. DNA samples from qPCR at saturation were analyzed by electrophoresis in a 1.5 % agarose gel. Cells carried an empty sgRNA vector (EV) or a vector expressing the sgRNA for the 1363 target in the *HO* promoter. Some marker fragment sizes in the NEB Quick-Load 50 bp DNA ladder are shown on the left, in base pairs. Bands were excised from the gel and subjected to DNA sequencing. Bands 1, 2 and 3 from the EV samples were all artifacts with sequences corresponding to portions of the yeast ribosomal precursor RNA. Band 1 from the 1363 samples showed only the expected sgRNA sequence. Values below each lane are the levels of RNA as measured by qPCR relative to the 0 minute time point. Thus, sgRNA was present at a low level prior to induction with doxycycline and was strongly induced at later times.

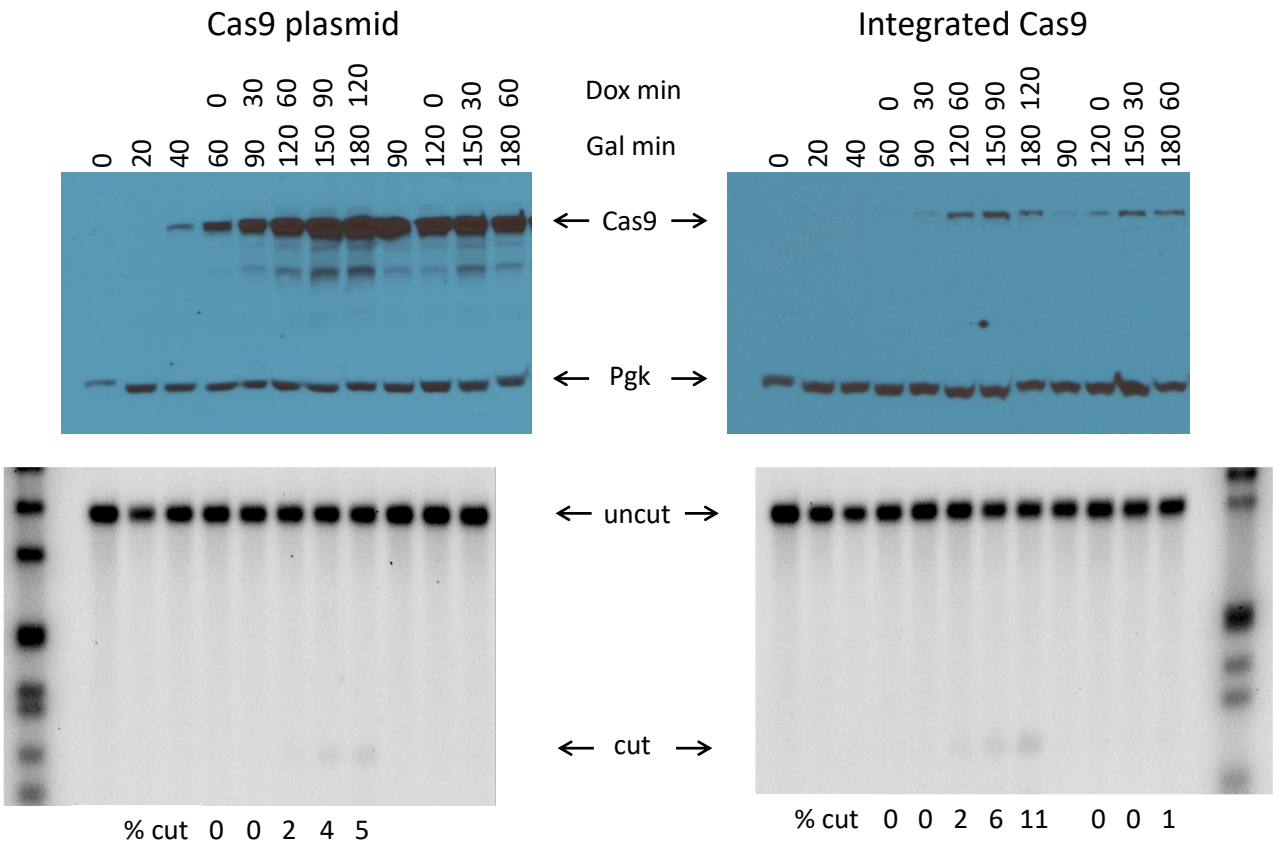


Fig. S3. Cleavage efficiency does not correlate with the level of Cas9 expression. Galactose-inducible Cas9 was expressed either from a low-copy plasmid (left) or an integrated gene (right). Western blots (top) show time courses of expression of Cas9 protein. Southern blots show cleavage mediated by the *HO* 1339 sgRNA at the same time points. In samples on the left of each image, sgRNA expression was induced by doxycycline addition 60 minutes after Cas9 induction with galactose. In the four samples on the right, doxycycline was added 120 minutes after galactose. Neither the apparent level of Cas9 protein nor the timing of sgRNA induction by doxycycline had a significant effect on cleavage efficiency. The *GAL* promoters on the plasmid and integrated Cas9 genes were essentially identical.

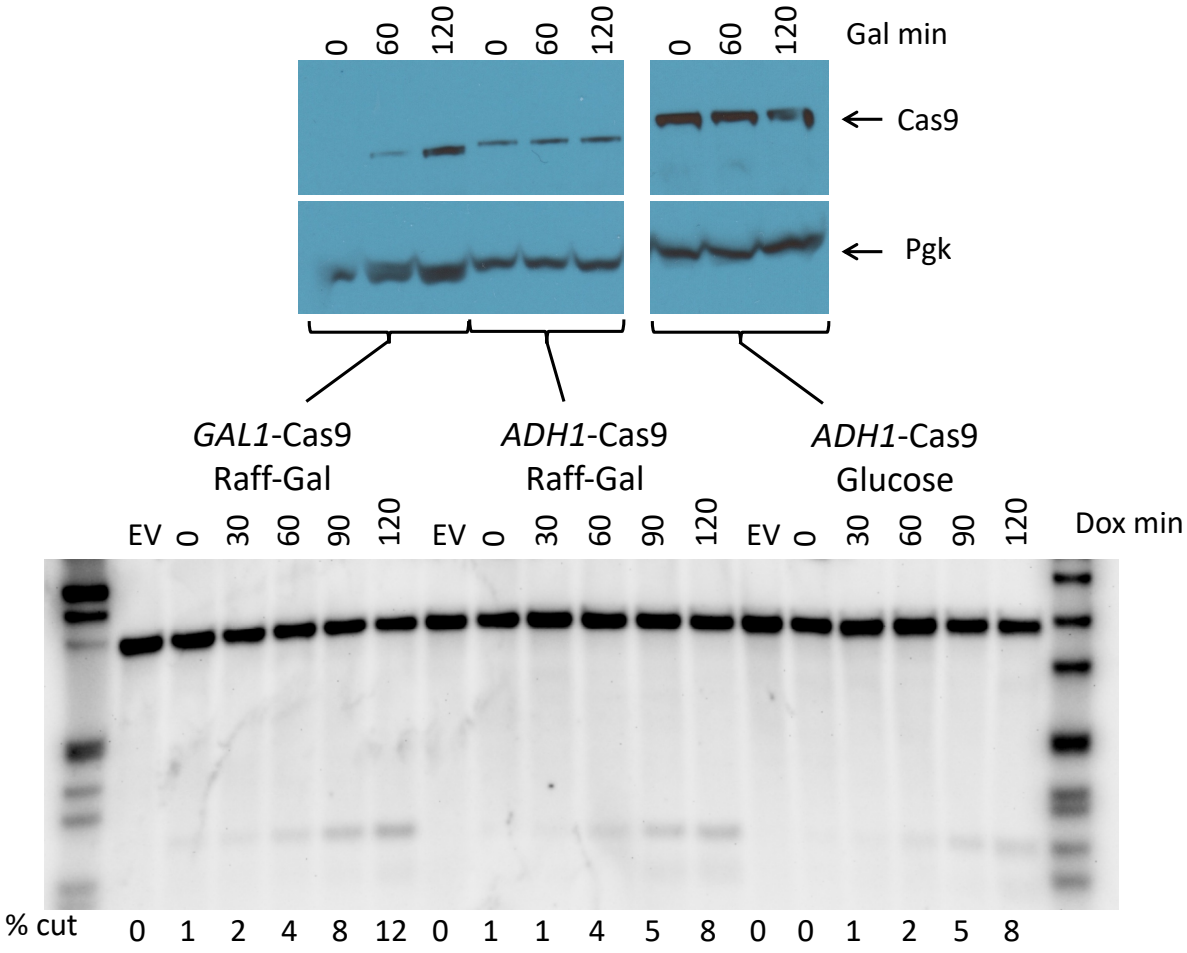


Fig. S4. Comparable levels of cleavage were seen with integrated Cas9 expressed from a *GAL1* promoter and from a constitutive *ADH1* promoter. A Western blot (top) shows expression levels of Cas9 protein after growth in raffinose and at various times after galactose induction (left) and a parallel sample grown in glucose (right). *GAL1*-Cas9 protein shows an increase with time, while the *ADH1*-Cas9 level was constant. The Southern blot (bottom) shows levels of cleavage for comparable samples in a time course after doxycycline induction of the *HO* 1363 sgRNA. Cleavage efficiency was slightly lower with the *ADH1*-Cas9.

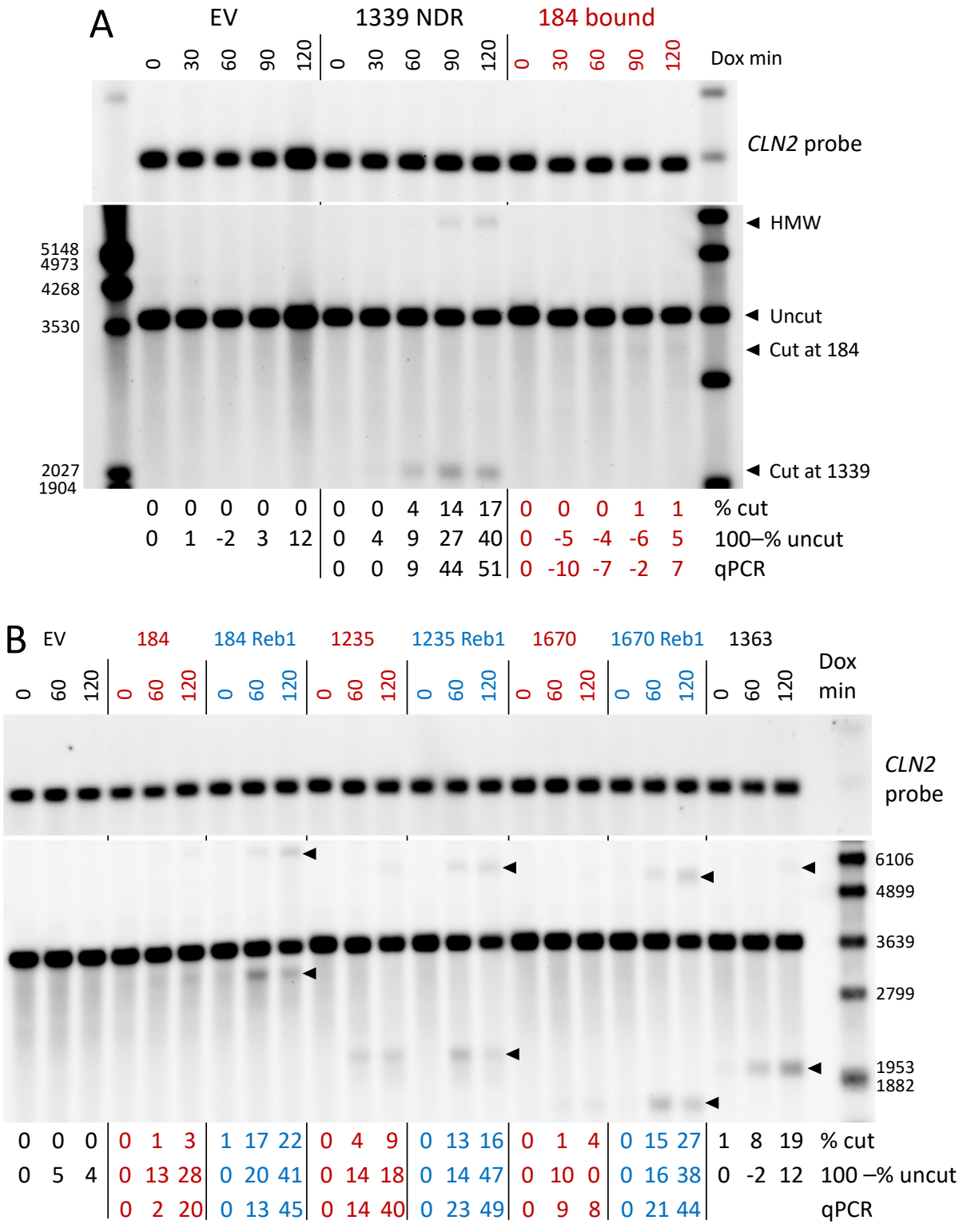


Fig. S5. Quantitation of the Southern blots in Figs. 1B (A) and 2C (B) by three methods. In each case, the result of stripping the blot and rehybridizing with a

probe for the *CLN2* promoter is shown. Using Fiji software, rows labeled “% cut” for each lane were quantitated by adding the intensity of the cut band and twice the intensity of the HMW band and dividing by that sum plus the intensity of the uncut band. In the rows labeled “100–% uncut” the ratio of the intensity of the uncut band in each lane was divided by the intensity of the corresponding *CLN2* band and normalized to the 0 minute time point for each target. “qPCR” reflects the loss of amplifiable (i.e., uncut) target DNA, normalized to *RPR1* in each sample and to the 0 minute time point; the EV and 1363 samples were not quantitated by qPCR. Some marker fragment sizes are shown, in base pairs.

In samples where the % cut is low, the values based on loss of uncut target are variable and sometimes negative. When the % cut is high, the 100–% uncut values are substantially higher. This likely reflects loss of hybridizable material in those samples that cannot be detected as bands. The differences between nucleosome-bound and NDR sites and between WT and Reb1 sites are retained regardless of the quantitation method.

In the right-most lanes in panel B, the failure of the 100–% uncut approach to report higher levels of cleavage may be due to non-uniform hybridization in that region with the *CLN2* probe.

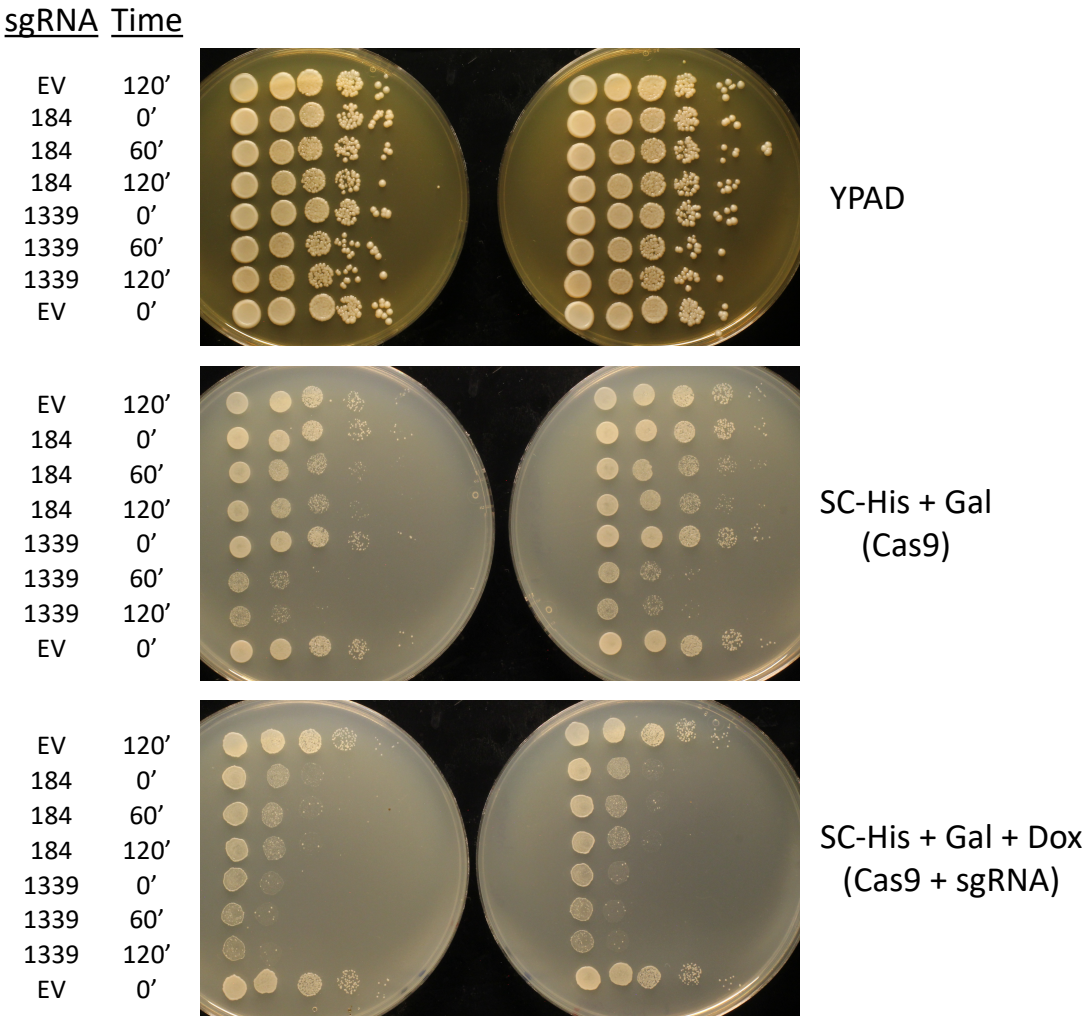


Fig. S6. Viability assays. Cultures were treated as for cleavage assays. Cas9 expression was induced in all samples; sgRNAs were induced with doxycycline (Dox) for the times indicated in liquid culture. Zero-time samples received no Dox. Ten-fold dilutions of these cultures were spotted onto the indicated plates: YPAD, rich medium; SC-His + Gal, selects for the sgRNA plasmid and continues Cas9 induction with galactose; SC-His + Gal + Dox, as above, but with continuing induction of sgRNA.

All samples grew well on YPAD (top), but there appears to be a slight deficit in the induced 1339 sgRNA cells, presumably due to the cleavage that had occurred in liquid culture. Given the levels of cleavage measured in those samples (≤50%) and the possibility of repair, extensive loss of viability is not

expected. When the sgRNA was induced in culture, viability was reduced with the continued expression of Cas9 (middle), presumably due to perdurance of the sgRNA. The effect on viability is more pronounced with the 1339 sgRNA than with the 184 sgRNA. With continued expression of both Cas9 and sgRNA (bottom), all the sgRNA-carrying cells show sharply reduced viability, perhaps somewhat more severe with 1339 sgRNA.

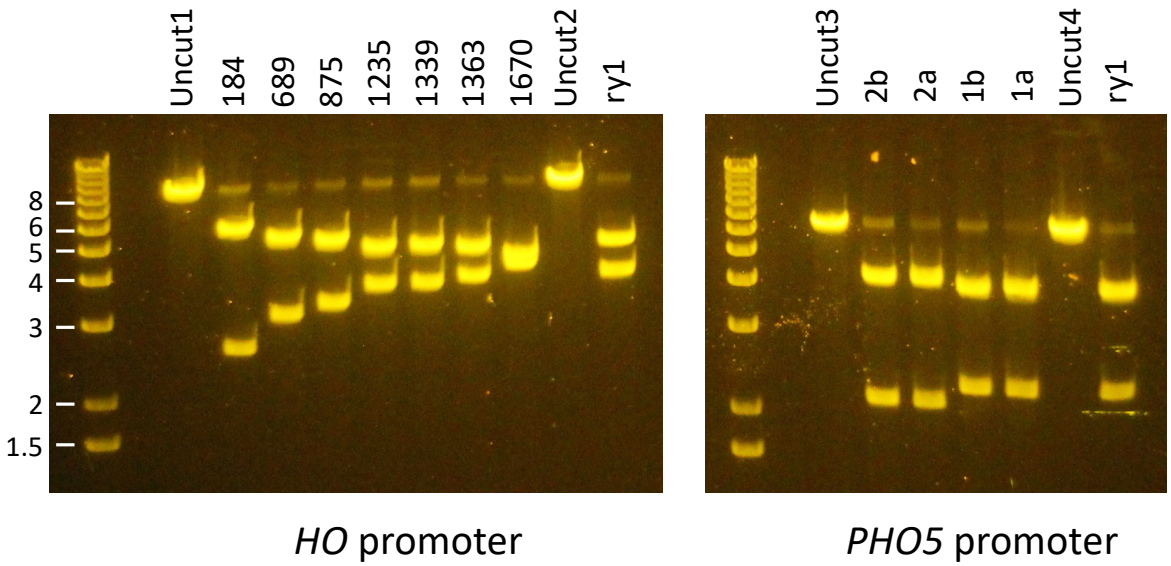
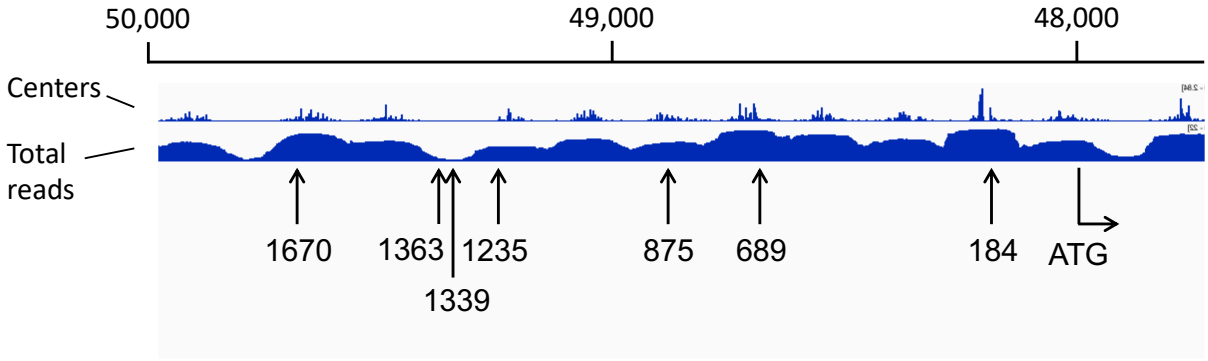


Fig. S7. Biochemical assays of Cas9 cleavage with sgRNAs used in these studies. Cas9 was assembled with each of the indicated sgRNAs and incubated with the appropriate linearized plasmid DNA substrate as described in Materials and Methods. The uncut samples are: Uncut1, wild type *HO* promoter (total length, 8.8 kb); Uncut2, *HO* promoter with the ry substitution at 1339 (8.8 kb); Uncut3, wild type *PHO5* promoter (6.65 kb); Uncut4, *PHO5* promoter with the ry substitution at 1a (6.65 kb). Sizes of marker fragments in the Invitrogen 1 Kb plus ladder are indicated on the left, in kb. The ratio of Cas9-sgRNA to DNA substrate is quite high in these assays, so they do not reflect relative activities of the various sgRNAs. Rather they are intended to show that all of the sgRNAs are functional.



S. cerevisiae chromosome IV: 50,000-47,750

Fig. S8. Nucleosome occupancy at the *HO* promoter determined by micrococcal nuclease mapping. The locations of the translation start (ATG) and the various sgRNA targets are indicated. The NDR in which the 1339 and 1363 targets are located is apparent. The level of nucleosome occupancy at the 875 and 1235 targets is clearly lower than at the other bound sites (184, 689, 1670). Data from McCullough, L, Pham, T.H., Chandrasekharan, M.B., Parnell, T.J., Stillman, D.J., Formosa, T. Manipulation of histone H3-K56 Acetylation reveals transcription-independent roles for FACT in establishing chromatin architecture, manuscript in preparation.

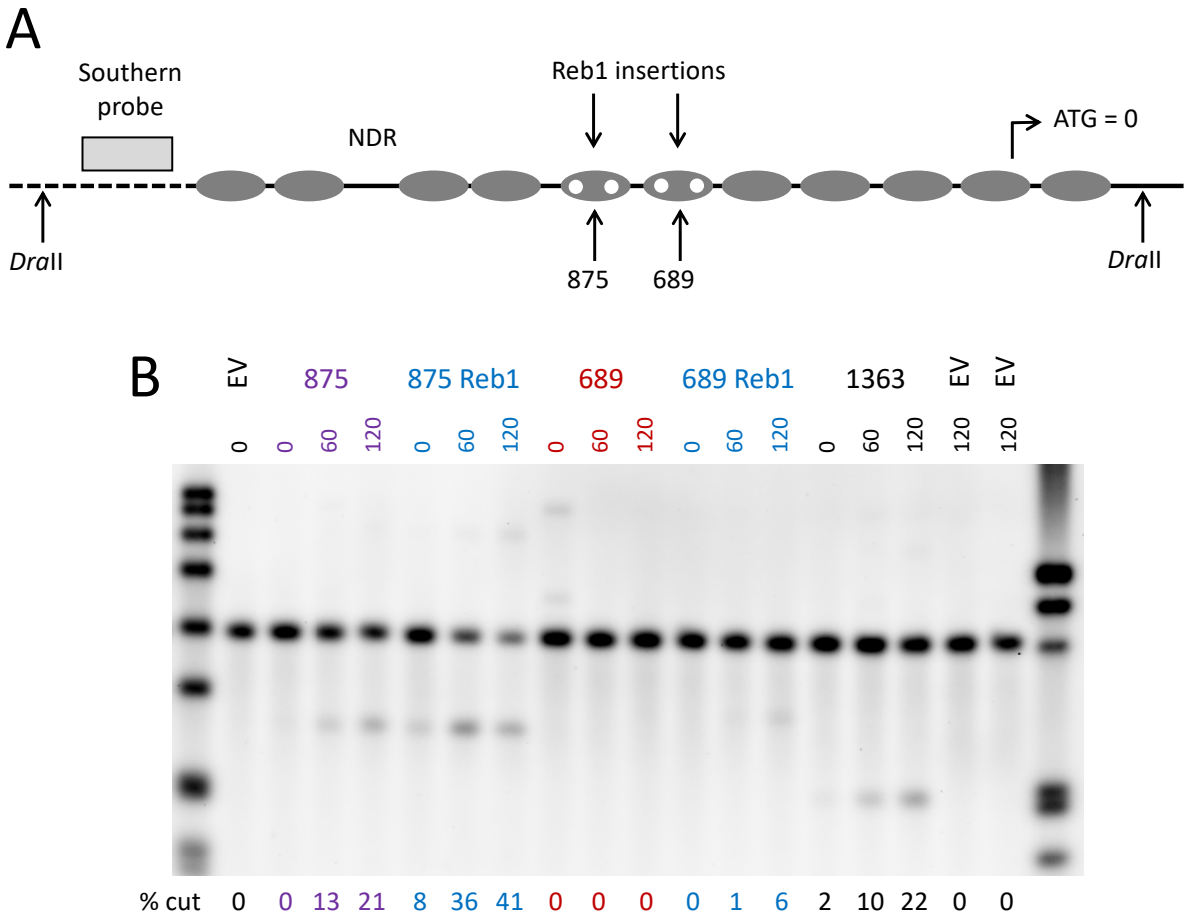


Fig. S9. Enhancement of cleavage at the *HO* 875 and 689 targets in the Reb1 site insertions. (A) Diagram of the paired Reb1 site insertions, each in a separate strain. (B) Southern blot showing Cas9 cleavage at 875 and 689 in wild type and Reb1 strains. Color scheme as in Figs. 1C and 2C. The empty vector samples are, from left to right, for the WT strain, 875 Reb1 and 689 Reb1.

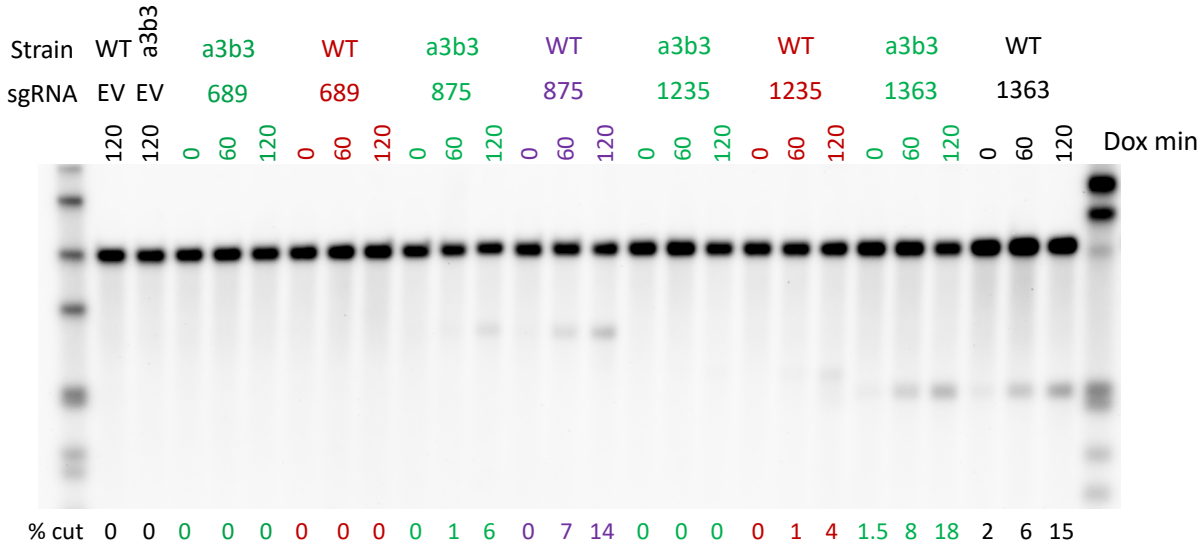


Fig. S10. Southern blot showing cleavage at the *HO* promoter 689, 875, 1235 and 1363 targets in the a3b3 strain, in which natural nucleosome eviction is suppressed by mutation of two Swi5 binding sites. There was little or no effect on the poorly cut 689 target or the robustly cut 1363 target, neither of which is in an area affected by Swi5 binding. Cleavage at both 875 and 1235 was markedly reduced in the a3b3 strain. Color scheme as in Figs. 1C and 2C, plus a3b3 in green.

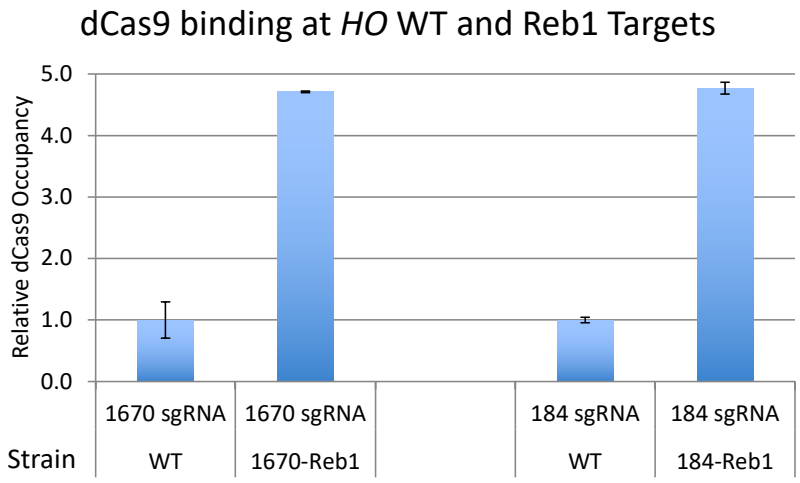
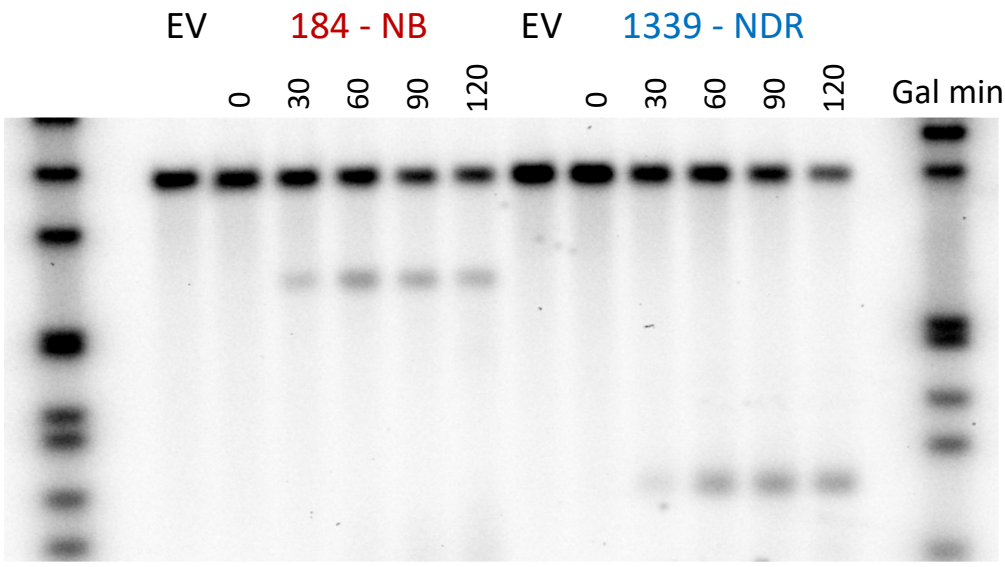


Fig. S11. Binding of dCas9 to the 184 and 1670 targets without and with Reb1 insertions. Values in the Reb1 strains are presented relative to WT, and error bars represent standard deviations of at least four measurements from two biological replicates.



% cut	0	0	10	21	22	19	0	0	9	22	30	36
100-% uncut	0	0	7	14	21	34	0	0	-4	17	34	55

Fig. S12. Time course of ZFN cleavage at the *HO* 184 and 1339 targets. Cleavage is seen at 30 minutes after nuclease induction by galactose, sooner than typically observed with Cas9 after sgRNA induction. Digestion of genomic DNA for this blot was done with *Nde*I + *Ngo*MIV rather than *Dra*II. ZFN cutting was stronger in this experiment than in the one shown in Fig. 6C. Two methods of quantitating the level of cleavage are shown.

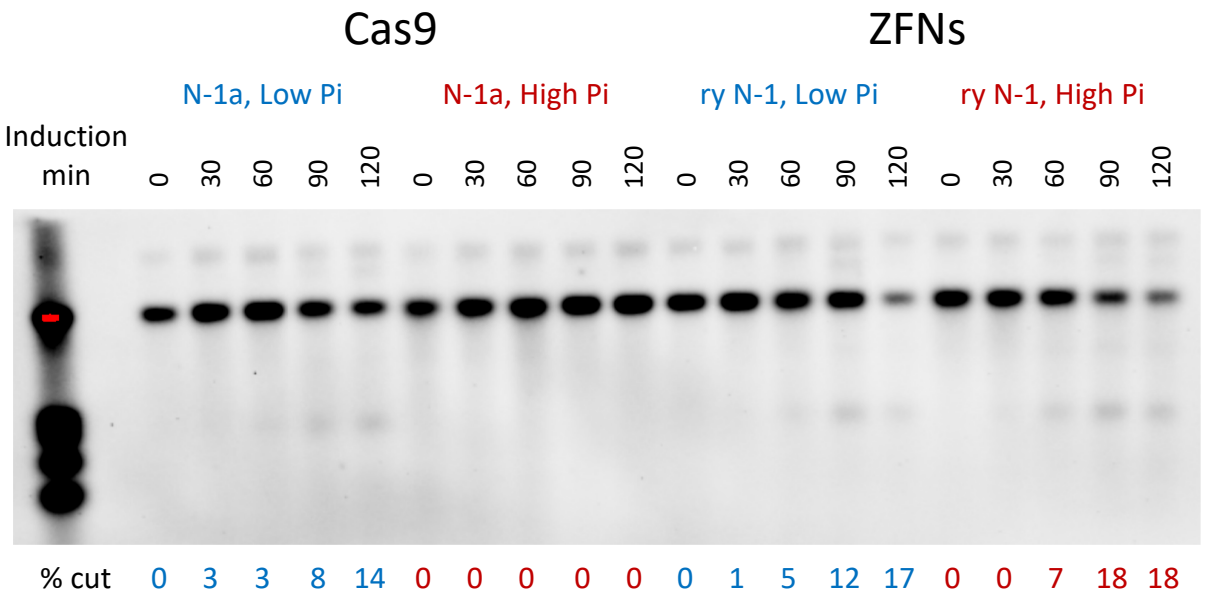


Fig. S13. Southern blot showing Cas9 and ZFN cleavage at the -1 nucleosome in the PHO5 promoter in low and high phosphate media. The Cas9 results were obtained in a wild type strain; those for the ZFNs in a strain with a ry replacement at the 1a site. Times in minutes are those following doxycycline addition for Cas9 and galactose addition for ZFNs.

Supplementary Tables

Table S1. Yeast strains

Strain	**Genotype**	**Plasmid**	**Notes**
FIG 1			
DCY136	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC037	
DCY138	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC038	
DCY140	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC039	
DCY142	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC036	
DCY270	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC040	
DCY271	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC041	
DCY272	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC042	
DCY280	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC060	
FIG 2			
DCY136	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC037	
DCY138	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC038	
DCY142	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC036	
DCY271	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC041	
DCY272	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC042	
DCY309	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−240 to −232 deleted]:TTTACCCG HO[−160 to −152 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC037	Reb1
DCY311	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1708 to −1700 deleted]:TTTACCCG HO[−1628 to −11620 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC042	Reb1

DCY313	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1268 to −1261 deleted]:TTACCCG HO[−1194 to −1187 deleted]:TTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC038	Reb1
FIG 3			
DCY136	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC037	
DCY138	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC038	
DCY270	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC040	
DCY271	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC041	
DCY272	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC042	
DCY280	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC060	
DCY309	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−240 to −232 deleted]:TTTACCCG HO[−160 to −152 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC037	Reb1
DCY311	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1708 to −1700 deleted]:TTTACCCG HO[−1628 to −11620 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC042	Reb1
DCY313	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1268 to −1261 deleted]:TTACCCG HO[−1194 to −1187 deleted]:TTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC038	Reb1
DCY450	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1818 to −1812 deleted]:TATTCC HO[−1309 to −1299 deleted]:TCGTATCATT ade2 can1 his3 leu2 trp1 ura3*	MC040	a3b3
DCY452	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1818 to −1812 deleted]:TATTCC HO[−1309 to −1299 deleted]:TCGTATCATT ade2 can1 his3 leu2 trp1 ura3*	MC060	a3b3
DCY454	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1818 to −1812 deleted]:TATTCC HO[−1309 to −1299 deleted]:TCGTATCATT ade2 can1 his3 leu2 trp1 ura3*	MC038	a3b3
DCY456	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1818 to −1812 deleted]:TATTCC HO[−1309 to −1299 deleted]:TCGTATCATT ade2 can1 his3 leu2 trp1 ura3*	MC041	a3b3
DCY460	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−725 to −717 deleted]:TTTACCCG HO[−671 to −663 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC040	a3b3

DCY464	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−913 to −905 deleted]:TTTACCCG HO[−838 to −830 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC060	Reb1
FIG 4			
DCY146	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC051	
DCY148	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC050	
DCY201	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*	MC036	
DCY202	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*	MC036	
FIG 5			
DCY203	*MAT***a** *PHO85(F82G) ade2 can1 his3 leu2 trp1 ura3*	MC036	
DCY204	*MAT***a** *PHO85(F82G) ade2 can1 his3 leu2 trp1 ura3*	MC036	
DCY233	*MAT***a** *URA3::GALp::yCas9::HphMX PHO85(F82G) ade2 can1 his3 leu2 trp1 ura3*	MC036	*pho85 (F82G)*
DCY239	*MAT***a** *URA3::GALp::yCas9::HphMX PHO85(F82G) ade2 can1 his3 leu2 trp1 ura3*	MC049	*pho85 (F82G)*
DCY241	*MAT***a** *URA3::GALp::yCas9::HphMX PHO85(F82G) ade2 can1 his3 leu2 trp1 ura3*	MC048	*pho85 (F82G)*
DCY243	*MAT***a** *URA3::GALp::yCas9::HphMX PHO85(F82G) ade2 can1 his3 leu2 trp1 ura3*	MC051	*pho85 (F82G)*
DCY245	*MAT***a** *URA3::GALp::yCas9::HphMX PHO85(F82G) ade2 can1 his3 leu2 trp1 ura3*	MC050	*pho85 (F82G)*
FIG 6			
DCY142	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC036	
DCY157	*MAT***a** *HO[−202 to −178 deleted]:AGCTACTACACGAATGGCGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	M3844, MC036	ry
DCY159	*MAT***a** *HO[−202 to −178 deleted]:AGCTACTACACGAATGGCGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC030, MC031	ry
DCY161	*MAT***a** *HO[−1346 to −1322 deleted]:AGCTACTACACGAATGGCGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	M3844, MC036	ry
DCY163	*MAT***a** *HO[−1346 to −1322 deleted]:AGCTACTACACGAATGGCGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC030, MC031	ry

DCY434	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−202 to −178 deleted]:AGCTACTACACGAATGGCGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC044	ry
DCY438	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−202 to −178 deleted]:AGCTACTACACGAATGGCGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC036	ry
DCY440	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1346 to −1322 deleted]:AGCTACTACACGAATGGCGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC044	ry
DCY444	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1346 to −1322 deleted]:AGCTACTACACGAATGGCGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC036	ry
DCY446	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1268 to −1261 deleted]:TTACCCG HO[−1194 to −1187 deleted]:TTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC036	Reb1
FIG 7			
DCY408	*MAT***a** *PHO85(F82G) ade2 can1 his3 leu2 trp1 ura3*	M3844, MC036	*pho85 (F82G)*
DCY410	*MAT***a** *PHO85(F82G) ade2 can1 his3 leu2 trp1 ura3*	M3844, MC036	*pho85 (F82G)*
DCY412	*MAT***a** *PHO85(F82G) PHO5:[-68 to -92}::AGCTACTACACGAATGGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	M3844, MC036	ry *pho85 (F82G)*
DCY414	*MAT***a** *PHO85(F82G) PHO5:[-68 to -92}::AGCTACTACACGAATGGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	M3844, MC036	ry *pho85 (F82G)*
DCY416	*MAT***a** *PHO85(F82G) PHO5:[-68 to -92}::AGCTACTACACGAATGGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC030, MC031	ry *pho85 (F82G)*
DCY549	*MAT***a** *URA3::GALp::yCas9::HphMX PHO85(F82G) PHO5:[-68 to -92}::AGCTACTACACGAATGGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC036	ry *pho85 (F82G)*
DCY551	*MAT***a** *URA3::GALp::yCas9::HphMX PHO85(F82G) PHO5:[-68 to -92}::AGCTACTACACGAATGGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC050	ry *pho85 (F82G)*
DCY553	*MAT***a** *URA3::GALp::yCas9::HphMX PHO85(F82G) PHO5:[-68 to -92}::AGCTACTACACGAATGGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC044	ry *pho85 (F82G)*
FIG S2			
DCY142	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC036	

DCY271	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC041	
FIG S3			
DCY266	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*	MC014, MC039	
DCY267	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	M3844, MC039	
FIG S4			
DCY142	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC036	
DCY271	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC041	
DCY298	*MAT***a** *URA3::ADH1::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC036	
DCY300	*MAT***a** *URA3::ADH1::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC041	
FIG S5			
DCY136	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC037	
DCY138	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC038	
DCY140	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC039	
DCY142	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC036	
DCY271	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC041	
DCY272	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC042	
DCY309	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−240 to −232 deleted]:TTTACCCG HO[−160 to −152 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC037	Reb1
DCY311	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1708 to −1700 deleted]:TTTACCCG HO[−1628 to −11620 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC042	Reb1
DCY313	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1268 to −1261 deleted]:TTACCCG HO[−1194 to −1187 deleted]:TTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC038	Reb1
FIG S6			

DCY466	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC063	
DCY468	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC064	
DCY470	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC065	
FIG S9			
DCY142	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC036	
DCY270	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC040	
DCY271	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC041	
DCY280	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC060	
DCY458	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−725 to −717 deleted]:TTTACCCG HO[−671 to −663 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC036	Reb1
DCY460	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−725 to −717 deleted]:TTTACCCG HO[−671 to −663 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC040	Reb1
DCY462	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−913 to −905 deleted]:TTTACCCG HO[−838 to −830 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC036	Reb1
DCY464	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−913 to −905 deleted]:TTTACCCG HO[−838 to −830 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	MC060	Reb1
FIG S10			
DCY138	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC038	
DCY142	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC036	
DCY270	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC040	
DCY271	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC041	
DCY280	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC060	
DCY348	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1818 to −1812 deleted]:TATTCC HO[−1309 to −1299 deleted]:TCGTATCATT ade2 can1 his3 leu2 trp1 ura3*	MC036	a3b3

DCY450	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1818 to −1812 deleted]:TATTCC HO[−1309 to −1299 deleted]:TCGTATCATT ade2 can1 his3 leu2 trp1 ura3*	MC040	a3b3
DCY452	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1818 to −1812 deleted]:TATTCC HO[−1309 to −1299 deleted]:TCGTATCATT ade2 can1 his3 leu2 trp1 ura3*	MC060	a3b3
DCY454	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1818 to −1812 deleted]:TATTCC HO[−1309 to −1299 deleted]:TCGTATCATT ade2 can1 his3 leu2 trp1 ura3*	MC038	a3b3
DCY456	*MAT***a** *URA3::GALp::yCas9::HphMX HO[−1818 to −1812 deleted]:TATTCC HO[−1309 to −1299 deleted]:TCGTATCATT ade2 can1 his3 leu2 trp1 ura3*	MC041	a3b3
FIG S11			
DCY561	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*	M5771, MC036	
DCY562	*MAT***a** *HO[−1708 to −1700 deleted]:TTTACCCG HO[−1628 to −11620 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	M5771, MC042	Reb1
DCY563	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*	M5771, MC036	
DCY564	*MAT***a** *HO[−240 to −232 deleted]:TTTACCCG HO[−160 to −152 deleted]:TTTACCCG ade2 can1 his3 leu2 trp1 ura3*	M5771, MC037	Reb1
FIG S12			
DCY157	*MAT***a** *HO[−202 to −178 deleted]:AGCTACTACACGAATGGCGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	M3844, MC036	ry
DCY159	*MAT***a** *HO[−202 to −178 deleted]:AGCTACTACACGAATGGCGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC030, MC031	ry
DCY161	*MAT***a** *HO[−1346 to −1322 deleted]:AGCTACTACACGAATGGCGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	M3844, MC036	ry
DCY163	*MAT***a** *HO[−1346 to −1322 deleted]:AGCTACTACACGAATGGCGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC030, MC031	ry
FIG S13			
DCY144	*MAT***a** *URA3::GALp::yCas9::HphMX ade2 can1 his3 leu2 trp1 ura3*	MC049	

DCY167	*MAT***a** *PHO5:[-68 to -92}::AGCTACTACACGAATGGCGTGGGA ade2 can1 his3 leu2 trp1 ura3*	MC030, MC031	ry

Table S2. Plasmids

Plasmid	**Description**	**Yeast ORI**	**Selection**
M2817	3.5kb HO promoter sequence cloned into pUC18	N/A	Amp
M3844	pRS415-Gal1 plasmid	CEN	LEU2
M5771	Adh1p:proteinA:dCas9 fusion expression vector	2µ	TRP1
MC030	Galp:RyA yeast plasmid	CEN	URA3
MC031	Galp:RyB yeast plasmid	CEN	LEU2
MC032	Galp:yCas9 integrating plasmid at *URA3*	N/A	KanMx
MC036	Tet-ON guide RNA plasmid, used as EV	CEN	URA3
MC037	Tet-ON guide RNA plasmid targeting *HO* -184	CEN	URA3
MC038	Tet-ON guide RNA plasmid targeting *HO* -1235	CEN	URA3
MC039	Tet-ON guide RNA plasmid targeting *HO* -1339	CEN	URA3
MC040	Tet-ON guide RNA plasmid targeting *HO* -706	CEN	URA3
MC041	Tet-ON guide RNA plasmid targeting *HO* -1363	CEN	URA3
MC042	Tet-ON guide RNA plasmid targeting *HO* -1670	CEN	URA3
MC044	Tet-ON guide RNA plasmid targeting ry sequence	CEN	URA3
MC048	Tet-ON guide RNA plasmid targeting *PHO5* 1b	CEN	URA3
MC049	Tet-ON guide RNA plasmid targeting *PHO5* 1a	CEN	URA3
MC050	Tet-ON guide RNA plasmid targeting *PHO5* 2b	CEN	URA3
MC051	Tet-ON guide RNA plasmid targeting *PHO5* 2a	CEN	URA3
MC058	PHO5 promoter cloned into pCR4-TOPO	N/A	Amp
MC059	NsiI to AflII HO promoter sequence containing 1339 ry sequence cloned into M2817	N/A	Amp
MC060	Tet-ON guide RNA plasmid targeting *HO* -875	CEN	URA3
MC061	PHO5 promoter with -1 ry cloned into pCR4-TOPO	N/A	Amp
MC063	Tet-ON guide RNA plasmid, used as EV	CEN	HIS3
MC064	Tet-ON guide RNA plasmid targeting *HO* -184	CEN	HIS3
MC065	Tet-ON guide RNA plasmid targeting *HO* -1339	CEN	HIS3

Table S3. Target sequences for sgRNAs

Promoter	**Target**	**Target sequence**	**PAM**	**Strand**
HO	184	GCTATTGCTACTCAAATG	AGG	top
	689	TGGTAGACGTGTGTGTCTCA	TGG	top
	875	TGAACCTGGTACGTATATTG	TGG	bottom
	1235	GATTATTTGATACCCCTTT	GGG	bottom
	1339	GATGTATCTCATCGCAGGCA	CGG	bottom
	1363	CAGTACAGTGCCCTGAGCGT	AGG	bottom
	1670	TGTTCTGGAGGCTTTACAAA	AGG	top
PHO5	1a	GCTGATGTTTTGCTAAGTCG	AGG	top
	1b	TTGCTAAGTCGAGGTTAGTA	TGG	top
	2a	GTCCCACGTGTGAGTGCCA	AGG	bottom
	2b	CAACCTTGGCACTCACACGT	GGG	top
ry target	ry1	AGCTACTACACGAATGGCGT	GGG	top

Table S4. PCR Primers

Oligo	**Description**	**Sequence**	**Position Relative to ATG**
PCR oligos			
DC089	Forward primer for *CLN2* Southern Probe	GGGTATCTGCGAATTGGAAA	-1568 to -1550
DC090	Reverse primer for *CLN2* Southern Probe	AGGAGCATAGAGGCGAATGA	-1100 to -1081
DC096	Forward primer for *PHO5* Southern Probe	GTTCCTTGGTTATCCCATCG	-990 to -971
DC097	Reverse primer for *PHO5* Southern Probe	CGTTGACATATTTGCGCATT	-571 to -498
DC116	Forward primer for *HO* Southern Probe	TTCAAAAGACGGTGCCATTA	-2273 to -2254
DC117	Reverse primer for *HO* Southern Probe	GGCACAATTTTACGTTGGAA	-1853 to -1834
DC235	Forward primer for *HO* -1339 ry cloning	CACGTAGTTCTTACTGGCAAAG	-1929 to -1908
DC236	Reverse primer for *HO* -1339 ry cloning	GTTACATCACTTTTCGTGACACA	-603 to -581
F3248	Forward primer for MX cassette conversion	GACATGGAGGCCCAGAATAC	
F3249	Reverse primer for MX cassette conversion	CGACAGCAGTATAGCGACCA	
RT-qPCR oligos			
DC136	Forward primer for sgRNA qPCR	ACTCGGTGCCACTTTTTCAA	
DC241	Reverse primer for sgRNA qPCR	CAGTACAGTGCCCTGAGCGT	
F1860	Forward primer for qPCR at *HO* -184	TGAATTGTACTACCGCTGGG	-287 to -268
F1862	Reverse primer for qPCR at *HO* -184	CGAAAAGTTCAACATAACTT	-247 to -228
F2087	Forward primer for qPCR at *HO* -1670	AAAGGCGGATCAAGATGTATGAAAG	-1742 to -1717
F2088	Reverse primer for qPCR at *HO* -1670	GGAACCATGTGATCTTACGTTGATATG	-1578 to -1551
F2091	Forward primer for qPCR at *HO* -1339	AAGCTAAGAATTTCACATGTTGTTG	-1471 to -1446

F2092	Reverse primer for qPCR at *HO* -1339	GTTGAGGTCTTTTCTATTTCTGATTG	-1276 to -1250
F2093	Forward primer for qPCR at *HO* -1235	AATGCTGGAGCAAAAATTTCAATCAG	-1295 to -1269
F2094	Reverse primer for qPCR at *HO* -1235	GGAGCCCCTCAGACATTAGCC	-1142 to -1121
F2153	Forward primer for qPCR of *PHO5* ORF	TTATTCTCGTGGTGTGCATTT	+775 to +795
F2154	Reverse primer for qPCR of *PHO5* ORF	CTTTAATAATTTGACTGAGGCATTG	+942 to +966
F2430	Forward primer for qPCR of *RPR1*	CACCTATGGGCGGGTTATCAG	
F2431	Reverse primer for qPCR of *RPR1*	CCTAGGCCGAACTCCGTGA	
ChIP-qPCR oligos			
DC160	Forward primer for ChIP at *PHO5* -2 nucleosome	TTTCGCATAGAACGCAACTG	-357 to -338
DC161	Reverse primer for ChIP at *PHO5* -2 nucleosome	ATGCCTTGCCAAGTAAGGTG	-171 to -152
DC213	Forward primer for ChIP at *PHO5* -56 to -278	GAATCGATACAACCTTGGCACT	-277 to -256
DC214	Reverse primer for ChIP at *PHO5* -56 to -278	TGAAGCCATACTAACCTCGACTT	-78 to -56
F1399	Forward primer for ChIP at IG-V control region	GGCTGTCAGAATATGGGGCCGTAGTA	
F1400	Reverse primer for ChIP at IG-V control region	CACCCCGAAGCTGCTTTCACAATAC	
F1860	Forward primer for ChIP at *HO* -184	TGAATTGTACTACCGCTGGG	-287 to -268
F1881	Reverse primer for ChIP at *HO* -184	AGTAGCAATAGCTGTTTACT	-207 to -188
F1883	Alternate Reverse primer for ChIP at *HO* -184	GCTTCATCATGCTTCAACAA	-167 to -148

F1908	Forward primer for ChIP at *HO* -1670	CGGATCAAGATGTATGAAAG	-1737 to -1718
F1928	Reverse primer for ChIP at *HO* -1670	GGATAAGATCGCACCTAACA	-1657 to -1638
F1960	Forward primer for ChIP at *HO* -875	AAAATATACACAAACGCCAC	-897 to -878
F1983	Reverse primer for ChIP at *HO* -875	AATCGACGACGGTCACATTA	-817 to -798
F1966	Forward primer for ChIP at *HO* -689	ATCTGACAACATGGTAGACG	-717 to -698
F1989	Reverse primer for ChIP at *HO* -689	CCCTTAAGCCCTGTGTAGGA	-637 to -618
F2001	Forward primer for ChIP at *HO* -1235	CCTCAACAGTAATTAACCCA	-1257 to -1238
F2005	Reverse primer for ChIP at *HO* -1235	TTTTACGCGATTCGGCCCAA	-1177 to -1158
F2087	Forward primer for dCas9 ChIP at *HO* -1670	AAAGGCGGATCAAGATGTATGAAAG	-1742 to -1717
F2088	Reverse primer for dCas9 ChIP at *HO* -1670	GGAACCATGTGATCTTACGTTGATATG	-1578 to -1551
F2091	Forward primer for ChIP at *HO* -1339	AAGCTAAGAATTTCACATGTTGTTG	-1471 to -1446
F2092	Reverse primer for ChIP at *HO* -1339	GTTGAGGTCTTTTCTATTTCTGATTG	-1276 to -1250
F2119	Forward primer for dCas9 ChIP at *HO* -184	ACCATTGGTACCTACTACTTTGAAT	-307 to -282
F2120	Reverse primer for dCas9 ChIP at *HO* -184	GCCATTTAGAATAGGAATTGAATAC	-100 to -75
F2647	Forward primer for ChIP at *PHO5* -1 nucleosome	CACCTTACTTGGCAAGGCATA	-171 to -150
F2648	Reverse primer for ChIP at *PHO5* -1 nucleosome	GTAATCTCGAATTTGCTTGCTCTATT	-28 to -2
F3201	Forward primer for ChIP at IG-1 control nucleosome	TGTCACGTAGGTAAAACACTTGC	
F3202	Reverse primer for ChIP at IG-1 control nucleosome	CCTTGATGGCGTGCTTAACT	

Reference

1. DeWitt MA*, et al.* (2016) Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells. *Science translational medicine* 8(360):360ra134.